The motivation for using two designs was to examine adaptation effects in two designs commonly used in fMRI: block designs (Grill-Spector et al., 1999) in which stimuli are repeated many times, and event-related designs (Kourtzi and Kanwisher, 2001) in which there is only one repetition of an image embedded within a long sequence of stimuli.

Sawamura and colleagues found that repeating the same image (AA or BB) produced maximal adaptation. Across neurons, they did not find a relation between the strength of adaptation and the level of initial signal. Instead, they found that the level of adaptation was constant for a neuron. This is consistent with a model suggesting that adaptation produces a proportional reduction of the neural responses (see fatigue model, Grill-Spector et al., 2006). Showing an image that the neuron was unresponsive to did not affect the response to a subsequent image that the neuron responded to (i.e., there was no adaptation). However, showing two images that the neuron responded to produced adaptation, but lesser than repeated presentation of an identical image. These data suggest that neural adaptation effects show higher sensitivity than the initial responses to the same stimulus.

What are the implications of the present study for the interpretation of fMRI-adaptation studies? (1) This study shows that there is cross-adaptation. Thus, two different stimuli that activate the same neuron will elicit some cross-adaptation. Conversely, if one finds fMRI-adaptation for a pair of stimuli it is likely that these two stimuli activate the same neurons. (2) Cross-adaptation in neurons was always smaller than adaptation by identical repeats. This is commonly found in fMRI-adaptation experiments, but see Kourtzi and Kanwisher (2001). (3) The effects of cross-adaptation in neural responses are more consistent with block fMRI-adaptation than short-lagged adaptation with a single repeat. This could be because of two reasons: (1) the mismatch between response-selectivity of neurons and neural adaptation decreases with repetition and (2) there are differential levels of neural adaptation and fMRI-adaptation for one stimulus repetition, but the level of neural adaptation and fMRI-adaptation are more similar following many repetitions (compare present study to Henson et al., 2004; Sayres and Grill-Spector, 2005).

Several questions remain open. Answering these questions will be crucial for understanding the neural mechanisms underlying adaptation (Grill-Spector et al., 2006). (1) Which neural population adapts most? Neurons that are optimally tuned to a stimulus, or neurons that are responsive, but not optimal? The current study shows that the initial level of response does not predict the level of adaptation, but Sawamura et al. did not examine whether the best stimulus from their set was also the optimal one. (2) What is the effect of adaptation when adapting a cell with its optimal stimulus compared to adaptation by a nonoptimal stimulus? For example, if the initial response to A is greater than that to B, will the adaptation level of B-A and A-B be similar or different? (3) Does adaptation affect the tuning width of neural receptive fields? For example, does adaptation make the tuning width narrower (as suggested by Wiggs and Martin, 1998)? (4) Is the relation between neural adaptation and selectivity similar for immediate (short-lag) adaptation and long-lag adaptation with many intervening stimuli between repeats? It is unknown whether the same neural mechanisms underlie immediate and long-lagged adaptation. However, fMRI researchers use both types of adaptation paradigms for inferring the functional properties of neural populations (e.g., Grill-Spector et al., 1999; Vuilleumier et al., 2002).

Future research that will link between monkey physiology, monkey fMRI, and human fMRI will provide the ultimate link in understanding effects across species (monkey and humans) and methods (single-unit recordings and fMRI). Sawamura and colleagues provide a critical stepping-stone.

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Selected Reading

Dobbins, I.G., Schnyer, D.M., Verfaellie, M., and Schacter, D.L. (2004). Nature *428*, 316–319.

Grill-Spector, K., and Malach, R. (2001). Acta Psychol. (Amst.) *107*, 293–321.

Grill-Spector, K., Kushnir, T., Edelman, S., Avidan, G., Itzchak, Y., and Malach, R. (1999). Neuron *24*, 187–203.

Grill-Spector, K., Henson, R., and Martin, A. (2006). Trends Cogn. Sci., in press. Published online November 28, 2005. 10.1016/j.tics.2005.11.006.

Henson, R.N., Rylands, A., Ross, E., Vuilleumeir, P., and Rugg, M.D. (2004). Neuroimage *21*, 1674–1689.

Kourtzi, Z., and Kanwisher, N. (2001). Science *293*, 1506–1509.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Nature *412*, 150–157.

Lueschow, A., Miller, E.K., and Desimone, R. (1994). Cereb. Cortex *4*, 523–531.

Mukamel, R., Gelbard, H., Arieli, A., Hasson, U., Fried, I., and Malach, R. (2005). Science *309*, 951–954.

Sawamura, H., Georgieva, S., Vogels, R., Vanduffel, W., and Orban, G.A. (2005). J. Neurosci. *25*, 4294–4306.

Sawamura, H., Orban, G.A., and Vogels, R. (2006). Neuron *49*, this issue, 307–318.

Sayres, R., and Grill-Spector, K. (2005). J. Neurophysiol., in press. Published online October 19, 2005. 10.1152/jn.00500.2005.

Schacter, D.L., and Buckner, R.L. (1998). Neuron *20*, 185–195.

Vuilleumier, P., Henson, R.N., Driver, J., and Dolan, R.J. (2002). Nat. Neurosci. *5*, 491–499.

Wiggs, C.L., and Martin, A. (1998). Curr. Opin. Neurobiol. *8*, 227–233.

DOI 10.1016/j.neuron.2006.01.004

Watching the Fly Brain Learn

The peptidergic dorsal paired medial (DPM) neurons, which innervate the mushroom bodies in *Drosophila*, have been widely hypothesized to be part of the unconditioned stimulus (US) pathway of odor-shock classical conditioning. In the December 2 issue of *Cell,* Yu et al., using functional imaging techniques, report the surprising finding that DPMs contain

odor-specific memory traces and send integrated information about the conditioned stimulus (CS) to the mushroom bodies. These findings provide important new insight into the circuitry of learning in *Drosophila.*

The goal of many early behavioral genetics researchers was to find "learning genes." These genes would be involved in processes unique to the formation of memory. As the field evolved and genetic screens were done in *Drosophila*, it looked very much like such genes had been found. But the cloning of these genes and the isolation of additional, often pleiotropic, alleles cast a shadow over the learning gene idea because many of the genes encoded proteins that had very general roles in cell function. Researchers quickly moved on to identifying the places where the learning genes were expressed to try and determine whether the localization of these gene products would specify circuits for memory in the brain. In one case, functional localization identified a very limited region—*amnesiac* (*amn*) expression in the fly adult brain was required in the two DPM neurons (Waddell et al., 2000). In other cases, localization studies pointed to the adult mushroom bodies as a likely venue for memory formation, but there were also many learning genes with spatially and temporally broad expression. Recent years have brought elegant studies that manipulate the function of candidate genes both in time and space, and the basic circuitry for several forms of learning has been worked out (cf. Joiner and Griffith, 1999; McGuire et al., 2004; Pascual and Preat, 2001; Zars et al., 2000). The addition of genetic tools to either kill or silence circuit components has allowed further refinement of these models.

In this way, *Drosophila* research has pushed the field of learning and memory into very molecular territory. The power of genetics has allowed the identification of many genes that have subsequently been shown to be critical to mammalian plasticity. So what is missing? Why do we not completely understand memory in the fly? Until recently, *Drosophila* has lagged sorrowfully behind big insects and many vertebrates in that it was not possible to routinely obtain functional information from the intact adult CNS. While fly people were piling up molecules and building virtual circuits, other neurobiologists were observing the behavior of neurons in real circuits, with electrodes and functional imaging. As demonstrated by the work of Yu and colleagues, and others, the melding of the genetic and functional approaches is likely to provide multiplicative gains in our understanding of plasticity (Riemensperger et al., 2005; Yu et al., 2004, 2005).

An important role for DPM neurons in associative memory was first suggested by the finding that the PACAP-like neuropeptides encoded by the *amn* gene were preferentially expressed in these cells (Waddell et al., 2000). *amn* mutants had long been known to have defects in the translation of short-term into medium-term memory (Quinn et al., 1979), and blockade of neurotransmission from DPMs phenocopied the *amn* mutant (Waddell et al., 2000). Further study revealed that blocking DPM output during the interval between training and testing disrupted memory assayed 3 hr after training, while blockade during training or retrieval had no effect (Keene et al., 2004). This phenotype, coupled with the fact that DPMs innervate the entire mushroom body neuropil made them an excellent candidate to be the bearer of reinforcing, or US, information in odor-shock learning. Peptidergic and aminergic neurons in a number of systems are believed to fulfill this role. Modulation in the *Aplysia* gill withdrawal circuit is carried out by both serotonin and peptides (Abrams et al., 1984). The bioamines octopamine (in insects) and dopamine (in insects and mammals) have been shown to be reinforcers (Hammer, 1997; Schultz, 2001; Schwaerzel et al., 2003).

To more directly test the hypothesis that DPMs signal the US, Yu and coworkers used state-of-the-art imaging technology to get a glimpse into this cell as the fly is learning. Using synapto-pHlorin, an activity-dependent marker of vesicle fusion, and G-CaMP, a calcium indicator, they asked the very simple question: what happens to the activity of the DPM neuron during memory formation? If it was part of the US input pathway to the mushroom body, this would predict activity regulated by shock but not odor, the CS. What they saw was a surprise. In naive animals, the DPM was responsive to electric shock, but it also responded robustly to every odor they tested. DPMs therefore are capable of responding to both the US and CS cues used in odor-shock classical conditioning.

To determine whether learning would modify DPM responses, Yu et al. applied a classical conditioning protocol to the animal during imaging. No changes were observed in odor responses 3 min after conditioning, a time when memory is unaffected by *amn* mutations. When the investigators looked at calcium influx 30 min after conditioning, however, they saw a CS-specific increase in the activity of the DPM with no change in the response to odors that had not been paired with electric shock. This change in activity lasted at least an hour, remarkably coincident with the temporal window in which they show that DPM activity is required for memory consolidation. The expression of this odor-specific increase in activity was dependent on *amn*, because it was absent in two independent alleles of *amn* but could be rescued by providing Amn to DPM with a transgene. An additional interesting observation made by this group was that the increase in activity was only seen in the vertical, α/α' lobes, while activity in the horizontal, $\beta/\beta'/\gamma$ lobes remained constant. There has been suggestion that α/α' is specifically involved in the formation of long-term memory (Pascual and Preat, 2001), and the finding that memory-related activity in DPM is directed at this structure is consistent with that idea.

But what does this tell us about the circuitry of associative learning in flies and the role of DPM? The first and most apparent thing is that DPM is not a "simple" modulatory neuron carrying a single channel of information into the mushroom body where it becomes integrated with information from other sensory channels (Figure 1, top). The second thing that is clear is that DPM is receiving information about the CS, odor, from an unknown source (Figure 1, bottom). Olfactory information in *Drosophila* is processed in the antennal lobes and goes out to the rest of the brain via projection neurons that are the invertebrate analogs of mitral cells. The projection neurons have clear inputs to the calyx of the

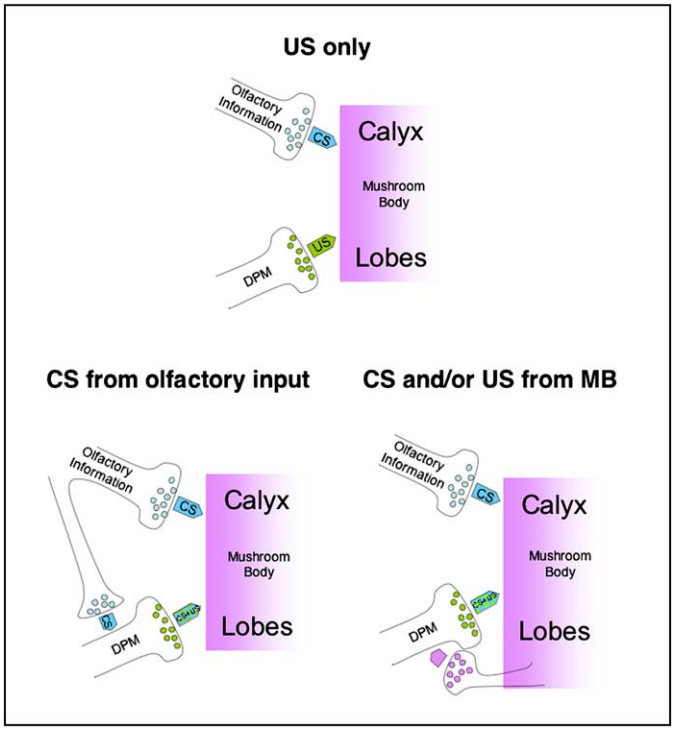

Figure 1. Models of DPM Function in the Adult *Drosophila* Brain

The top panel shows a previously prevailing model of DPM as a carrier of US information into the mushroom bodies. The bottom panel shows alternative models of DPM function suggested by the work of Yu et al. DPM may receive olfactory input and information about shock from non-Kenyon cell inputs and integrate this information (left). DPM may receive CS and/or US information in an integrated form from Kenyon cells and act as part of a stabilizing feedback loop.

mushroom bodies, where the Kenyon cell somata reside, and to cells in the rather ill-defined lateral horn. The anatomy of the DPM limits the possible site of inputs to either its soma or to its projection field in the mushroom body lobes. Because invertebrate neurons commonly have projections that contain both presynaptic and postsynaptic specializations, it is likely that odor information is being received within the mushroom bodies either from the Kenyon cells themselves or from other neurons that project into that neuropil.

The third and most interesting feature of DPMs revealed by this study is their relay of processed information about the specific CS-US pair into the mushroom body lobes. One attractive idea is that DPM might itself be an integrator of US and CS information and a repository of a memory trace. This would imply that DPM receives "raw" information about the CS (from olfactory inputs or mushroom body) and the US (perhaps via dopaminergic inputs into the lobes, Riemensperger et al., 2005) and does a computation. But the fact that an association-specific activity is present in DPM does not necessarily imply that DPM is itself an integrator. If the CS and US information comes into DPM from Kenyon cells, it is possible that association and the alteration in the strength of the signal might be a result of mushroom body processing. In this scenario, DPM is a follower—perhaps part of a positive-feedback loop that stabilizes the memory trace in the mushroom body. This second possibility is supported by Waddell's finding (Waddell et al., 2000) that 3 min memory is unaffected by blocking DPM output, suggesting that this cell does not have a role in the initial association of the US and CS.

How can these models be differentiated? Luckily there are genetic tools that can be used with live imaging to dissect the problem. If mushroom bodies are providing CS and/or US information to DPMs then disruption of neurotransmission in Kenyon cells should alter DPM responses to the CS and/or US cues. Previous experiments that silenced these cells during the DPM-requiring epoch of 3 hr memory did not detect a requirement for mushroom body activity at this time (McGuire et al., 2001), but it is possible that the subset of Kenyon cells inactivated in this study did not sufficiently diminish DPM input to block consolidation. Examining these and other candidate inputs to DPM by using imaging or direct recording is now feasible. Determining who is talking to DPM, and when, will be an important next step toward understanding the cellular basis of associative memory formation in *Drosophila.*

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Selected Reading

Abrams, T.W., Castellucci, V.F., Camardo, J.S., Kandel, E.R., and Lloyd, P.E. (1984). Proc. Natl. Acad. Sci. USA *81*, 7956–7960.

Hammer, M. (1997). Trends Neurosci. *20*, 245–252.

Joiner, M.A., and Griffith, L.C. (1999). Learn. Mem. *6*, 177–192.

Keene, A.C., Stratmann, M., Keller, A., Perrat, P.N., Vosshall, L.B., and Waddell, S. (2004). Neuron *44*, 521–533.

McGuire, S.E., Le, P.T., and Davis, R.L. (2001). Science *293*, 1330–1333.

McGuire, S.E., Mao, Z., and Davis, R.L. (2004). Sci. STKE, http://stke.sciencemag.org/cgi/content/full/OC_sigtrans;stke.2202004pl6.

Pascual, A., and Preat, T. (2001). Science *294*, 1115–1117.

Quinn, W.G., Sziber, P.P., and Booker, R. (1979). Nature *277*, 212–214.

Riemensperger, T., Voller, T., Stock, P., Buchner, E., and Fiala, A. (2005). Curr. Biol. *15*, 1953–1960.

Schultz, W. (2001). Neuroscientist *7*, 293–302.

Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., and Heisenberg, M. (2003). J. Neurosci. *23*, 10495–10502.

Waddell, S., Armstrong, J.D., Kitamoto, T., Kaiser, K., and Quinn, W.G. (2000). Cell *103*, 805–813.

Yu, D., Ponomarev, A., and Davis, R.L. (2004). Neuron *42*, 437–449.

Yu, D., Keene, A.C., Srivatsan, A., Waddell, S., and Davis, R.L. (2005). Cell *123*, 945–957.

Zars, T., Wolf, R., Davis, R., and Heisenberg, M. (2000). Learn. Mem. *7*, 18–31.

DOI 10.1016/j.neuron.2006.01.001